WEST Search History

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DATE: Monday, July 19, 2004

Hide?	Set Name	Query	Hit Count
	DB=USPT,DWPI; PLUR=YES; OP=ADJ		
☐	L1	erikson-G$.in. or Daksis-J$.in.	24
☐	L2	5030557	112
☐	L3	enhanc$ near10 hybridiz$	1489
☐	L4	hairpin near block$	10
☐	L5	hairpin same block$	421
☐	L6	probe same target same hybridiz$	8903
☐	L7	L6 and L3	858
☐	L8	L7 and L1	1
☐	L9	L7 and L5	23
☐	L10	6265170.pn. or 5770365.pn.	4
	DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ		
☐	L11	probe same target	33797
☐	L12	L1 same (hybridiz$)	0
☐	L13	L11 same (hybridiz$)	18187
☐	L14	L13 same (avidity or specificity)	2367
☐	L15	L14 same (helper or block$ or nucleobase)	94
☐	L16	l15 same enhanc$	16
☐	L17	l15 and hairpin	27
☐	L18	probe same (conjugat$ same (helper or block$))	885
☐	L19	L18 same (avidity or specificity)	37
☐	L20	L18 same hairpin	4
☐	L21	probe same (conjugat$ near (helper or block$) or nucleobase)	449
☐	L22	probe same (conjugat$ near (helper or block$ or nucleobase))	8
☐	L23	6312952.pn.	2
☐	L24	6312925.pn.	2
☐	L25	hairpin near (block$ or help$ or avidity)	27
☐	L26	hairpin near (help$ and avidity)	0
☐	L27	probe near (help$ and avidity)	0
☐	L28	probe same (enhanc$ near avidity)	2
☐	L29	hairpin same (enhanc$ near avidity)	0
☐	L30	probe and (enhanc$ near avidity)	70

☐	L31	L30 and hybridiz$	54
☐	L32	L31 and (hairpin near conformation$)	1
☐	L33	L31 and hairpin	2

END OF SEARCH HISTORY

L3 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:565002 CAPLUS
DOCUMENT NUMBER: 109:165002
TITLE: Dot blot detection of point mutations with adjacently hybridizing synthetic oligonucleotide probes
AUTHOR(S): Alves, A. M.; Carr, F. J.
CORPORATE SOURCE: Pharm. Div., ICI, Macclesfield/Cheshire, SK10 4TG, UK
SOURCE: Nucleic Acids Research (1988), 16(17), 8723
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A simple method is described that **enhances** the hybridization **specificity** of oligonucleotide probes for dot blot anal. Gels are hybridized with a 32P-labeled oligonucleotide **probe**, together with an adjacently hybridizing **nucleotide**, for 2 h. Then the gels are immersed in ligase solution (0.3 units/μL T4 DNA ligase) for 80 min. at 16° before washing. The ligase treatment resulted in the removal of the background high-mol.-weight hybridization signal and a specific dot blot hybridization of the probe to target DNA alone. This method was used to detect the activating mutation in the Ha-ras gene in human T24 bladder carcinoma cells.

=>

FILES 'MEDLINE, BIOTECHDS, EMBASE, BIOSIS, SCISEARCH, CANCERLIT, CAPLUS'
ENTERED AT 12:23:19 ON 19 JUL 2004

7 FILES IN THE FILE LIST

=> s (probe or hairpin) (10a) (block? or helP? or nucleobase or nucleoside or nucleotide)
L1 10321 (PROBE OR HAIRPIN) (10A) (BLOCK? OR HELP? OR NUCLEOBASE OR NUCLEOSIDE OR NUCLEOTIDE)

=> s l1 and (enhanc? (5a) (specificity or avidity))
L2 12 L1 AND (ENHANC? (5A) (SPECIFICITY OR AVIDITY))

=> dup rem l2
PROCESSING COMPLETED FOR L2
L3 11 DUP REM L2 (1 DUPLICATE REMOVED)

=> d ibib abs l3 1-11

L3 ANSWER 1 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-10612 BIOTECHDS
TITLE: Specifically detecting perfectly matched probe-target hybrids, by annealing hairpin-base probe to nucleic acid target to form perfectly matched probe-target hybrids, and electrochemically detecting annealed probe signal; Haemophilus influenzae NADH-dehydrogenase gene SNP detection using horseradish peroxidase-labelled DNA probe
AUTHOR: HO C; LIU M; MILLER J F; WANG T
PATENT ASSIGNEE: UNIV CALIFORNIA
PATENT INFO: WO 2003000917 3 Jan 2003
APPLICATION INFO: WO 2002-US19649 21 Jun 2002
PRIORITY INFO: US 2001-300235 21 Jun 2001; US 2001-300235 21 Jun 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-210158 [20]
AN 2003-10612 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Specifically detecting (M1) perfectly matched probe-target hybrids, comprises annealing a hairpin-base probe to a nucleic acid target under conditions favoring the formation of perfectly matched probe-target hybrids over mismatched probe-target hybrids, and electrochemically detecting annealed probe signal.
DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) Detecting (M2) an electrochemically detectable molecule, by amplifying molecular signal by catalyzed reporter deposition, and electrochemically detecting the amplified signal; (2) A system (I) for specifically detecting perfectly matched probe-target hybrids, comprises a hairpin-based probe for annealing to a nucleic acid target under conditions favoring the formation of perfectly matched probe-target hybrids over mismatched probe-target hybrids, and a sensor for electrochemically detecting annealed **probe** signal; (3) A system (II) for typing a single **nucleotide** polymorphism of a nucleic acid target sequence, comprising: (a) adding at least one additional hairpin-based probe to (I), each probe for annealing in a separate reaction and comprising a sequence complementary to the target sequence, where the complementary sequences of the probes differ from one another at a single **nucleotide** position, and one **probe** forms a perfectly matched hybrid with the target sequence; or (b) at least two hairpin-based detector probes for annealing in separate reactions to the target sequence under conditions favoring the formation of perfectly matched probe-target hybrids over mismatched probe-target hybrids, each probe comprising a sequence complementary to the target sequence, where

the complementary sequences of the probes differ from one another at a single **nucleotide** position, and one **probe** forms a perfectly matched hybrid with the target sequences, and a sensor for electrochemically detecting annealed probe by horse radish peroxidase-catalyzed reduction of an electrochemically active substrate; and (4) A system (III) for detecting an electrochemically detectable molecule, comprising an enzymatic system for amplifying molecular signal by catalyzed reporter deposition, and a sensor for electrochemically detecting amplified signal.

BIOTECHNOLOGY - Preferred Method: In M1, the probe is a detector probe, and is labeled with an electrochemically detectable molecule such as fluorescein. The probe is labeled at one end with an electrochemically detectable molecule and attached at the other end to a molecule inhibiting electrochemical detection of the electrochemically detectable molecule. The probe is labeled at one end with horse radish peroxidase and attached at the other end to benzhydroxamic acid. The annealing step involves incubating the probe and target in the presence of at least one hairpin-based competitor probe. M1 further comprises an amplification step after annealing and before detection, where the step comprises amplifying annealed probe signal by catalyzed reporter deposition of a tyramide conjugate. The annealed probe signal is electrochemically detected by horse radish peroxidase-catalyzed reduction of an electrochemically active substrate. Preferred System: (I) or (II) further comprises at least one hairpin-based competitive probe for incubating with the target, and an enzymatic system for amplifying annealed probe signal by catalyzed reporter deposition prior to electrochemical detection.

USE - M1 is useful for specifically detecting perfectly matched **probe**-target hybrids. M1 is useful for typing a single **nucleotide** polymorphism of a target sequence, by applying M1 to at least two hairpin-based probes, one probe forming a perfectly matched hybrid with the target sequence, each probe annealed in a separate reaction and comprising a sequence complementary to the target sequence, where the complementary sequences of the probes differ from one another at a single **nucleotide** position, or by annealing each of at least two **hairpin**-based detector probes in separate reactions to the target sequence under conditions favoring the formation of perfectly matched probe-target hybrids over mismatched probe-target hybrids, one probe forming a perfectly matched hybrid with the target sequence, each probe comprising a sequence complementary to the target sequence, where the complementary sequences of the probes differ from one another at a single **nucleotide** position, and electrochemically detecting annealed **probe** signal by horse radish peroxidase-catalyzed reduction of an electrochemically active substrate. M2 is useful for detecting an electrochemically detectable molecule such as a nucleic acid probe annealed to a nucleic acid target (claimed).

ADVANTAGE - M1 is potentially easier, less costly and more rapid than current single nucleotide polymorphism typing methods. M1 has enhanced sensitivity due to catalyzed reporter deposition of probe signal prior to electrochemical detection, and **enhanced specificity** due to inclusion of hairpin-based competitor probes in the annealing step.

EXAMPLE - The specificity of a hairpin-based probe and a linear probe were compared by sandwich DNA hybridization. A 46 base oligonucleotide exactly corresponding to bases 6262-6307 of the Haemophilus influenzae NADH dehydrogenase gene and having the sequence of gctaaagtaacgctggttgatcgcaatgccacccattt atggaaac was provided as a perfectly matched target, and a similar 46 base oligonucleotide gctaaagtaacgctggttgatcgcaatgcca ccgatttatggaaac with a single nucleotide difference was provided as a 1 base pair mismatched target. A 46 base pair oligonucleotide corresponding to bases 3962-4007 of the Streptococcus pneumonia penicillin binding protein 1b gene and having the sequence of ttatctcgggcaaaatggcgatacgcaaattc acggttttgaatta was provided as a negative control target. A fluorescein-labeled linear detector probe

(cataaatgggtggca) and a fluorescein-labeled hairpin-based detector probe (gcgagcataaatgggtggcactcgc), each having a sequence perfectly complementary to a region of the perfectly mismatched target sequence, were annealed to each target in separate reactions. The 1 base mismatch with mismatched target was located at the center of each detector probe. Hybridization and detection were performed as follows. A 65 microl aliquot of a nucleic acid target in 0.17 M NaOH was incubated at room temperature for 5 minutes. To the aliquot was added 25 microl of probe solution containing 200 nM biotin-labeled capture probe (tcaaccagcgttactttagc) and 100 nM detector probe in 1.05 M Tris, 0.5 M NaCl, 1 mM MgCl2 and 0.05% bovine serum albumin, pH 7.8. The mixture was incubated at 65degreesC for 10 minutes, then loaded onto an electrochemical sensor. Following a wash step using biotin wash solution, 50 microl of an horse radish peroxidase (HRP)-conjugated anti-fluorescein monoclonal antibody at a concentration of 0.75 U/ml was added to the sensor surface and incubated at room temperature for 10 minutes. After a wash step, the sensors were placed in a pulse amperometric monitor and 50 microl of a substrate (K-blue) was dispensed onto the sensor. Amperometric signals were determined 70 seconds after addition of substrate. The signals from the perfectly complementary hybrid (perfect match) and the 1 base mismatched hybrid (1 bp mismatch) of the linear probe were similar, indicating that the linear probe could not distinguish between perfectly matched and mismatched targets. In contrast, the signal from the perfectly matched hybrid of the hairpin-based probe was about ten-fold greater than the signal from the 1 base pair mismatched hybrid of the hairpin-based probe. Unlike the linear probe, the hairpin-based probe was able to distinguish between perfectly matched and mismatched targets. To further increase specificity, a hairpin-based competitor probe having a target-recognizing sequence in the loop was added to the hybridization mixture. A hairpin competitor probe with a mismatched base in the center of the loop sequence (gcgagcataaatcggtggcactcgc) was added to a sandwich DNA hybridization mixture containing either the perfectly matched target and the H.influenzae hairpin-based detector probe described above, or the 1 base pair mismatched target and the H.influenzae hairpin-based detector probe. Different ratios of competitor to detector probe were employed. The signal produced by the mismatched hybrid (1 bp mismatch) progressively decreased in the presence of increasing amounts of competitor probe, i.e., from a competitor to detector ratio of 1 (competitor:detector probe = 1) to a ratio of 10 (competitor:detector probe = 10). In contrast, the signal produced by the perfectly matched hybrids (perfect match) and negative control (negative) did not change significantly as the competitor to detector ratio was increased. (29 pages)

L3 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2003:173022 CAPLUS
DOCUMENT NUMBER: 138:216442
TITLE: System and method for assaying nucleic acid molecules using probes with unstructured nucleotide (UNA) to minimize cross hybridization
INVENTOR(S): Yakhini, Zohar H.; Sampson, Jeffrey R.; Myerson, Joel
PATENT ASSIGNEE(S): Agilent Technologies, Inc., USA
SOURCE: Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1288313	A2	20030305	EP 2002-255908	20020823
EP 1288313	A3	20040114		

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT,

IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK
US 2003211474 A1 20031113 US 2001-938937 20010824
PRIORITY APPLN. INFO.: US 2001-938937 A 20010824
AB The present invention provides a system and methods for assaying nucleic acid mols. with reduced levels of background signal and **enhanced specificity** and sensitivity. In particular, the present invention provides a system and methods for detecting, sorting, tracking and characterizing nucleic acid mols. using hybridization assays with reduced levels of undesirable cross hybridization and reduced levels of intramol. secondary structure. Nucleic acid mols. with reduced secondary structure ("unstructured nucleic acids"; UNA) are generated by enzymically incorporating modified nucleotide triphosphates that have a reduced ability to form base pairs with complementary modified and unmodified nucleotides. In a particularly preferred embodiment, unstructured nucleic acids are enzymically synthesized by incorporating triphosphate forms of 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolopyrimidine and combinations therein.

L3 ANSWER 3 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-06903 BIOTECHDS
TITLE: Detecting a target nucleic acid in a sample for high throughput genetic analysis by hybridizing probes to target nucleic acids;
DNA polymorphism detection using DNA probe useful for high throughput screening
AUTHOR: KNAPP M R; KOFP-SILL A R; SIMEONOV A; PEPONNET C
PATENT ASSIGNEE: CALIPER TECHNOLOGIES CORP
PATENT INFO: WO 2002083952 24 Oct 2002
APPLICATION INFO: WO 2002-US11712 11 Apr 2002
PRIORITY INFO: US 2001-283527 12 Apr 2001; US 2001-283527 12 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-093040 [08]
AN 2003-06903 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Detecting a target nucleic acid in a sample comprising providing at least a first and a second group of nucleic acid probes, hybridizing at least a first probe from the first group and at least a second probe from the second group to target nucleic acid, and detecting the hybridization by a non-Sanger detection step, is new.
DETAILED DESCRIPTION - Detecting a target nucleic acid in a sample comprising providing at least a first and a second group of nucleic acid probes, hybridizing at least a first probe from the first group and at least a second probe from the second group to target nucleic acid, and detecting the hybridization by a non-Sanger detection step, is new. The first group of probes comprises at least 10 % of all possible nucleic acid probes having x number of nucleotides and the second group of probes including at least 10 % of all possible nucleic acid sequences having at least y number of nucleotides. INDEPENDENT CLAIMS are also included for the following: (1) detecting a polymorphic variant in a polymorphic nucleic acid sequence; (2) a set of nucleic acid probes for detecting a target nucleic acid sequence in a sample comprising at least two groups of nucleic acid probes, the first or second of which comprises at least 10 % of all possible nucleic acid probe sequences having x or y nucleotides, respectively, where several members of each of the first and second groups are labeled; (3) a library of nucleic acids comprising at least 105 of all possible nucleic acids for a monomer length x, where x is at least 5; or (4) a genetic analysis system comprising: (a) a vessel comprising a mixture having the target nucleic acid; (b) several sources of nucleic acid probes, each of which includes probes of at least 10 % of all possible nucleic acid probe sequences of length x or y; and (c) a system for selectively delivering different probes from the sources of probes to the vessel, comprising: (a) system instructions that identify and select probes to be delivered to the vessel; and (b) a sampling

system for sampling and delivering probes from the sources of probes to the vessel, where the vessel is a microfluidic device and the system instructions select probes that are complementary to a region of interest on the target nucleic acid.

BIOTECHNOLOGY - Preferred Method: Detecting a target nucleic acid in a sample further comprises: (a) hybridizing at least a third probe from the third group to the target nucleic acid substantially proximal to at least one of the first and second probes; (b) ligating the first, second and third probes together with a ligase; (c) selecting the non-human animal or organism, plant, bacteria, fungi, archae or cell based upon detection of the target nucleic acid; and (d) selecting a treatment, diagnosing a disease or diagnosing a genetic predisposition to a disease based upon detection of the target nucleic acid. Detecting hybridization of the first or second probes comprises FRET detection. The detecting step comprises observing the fluorescence of the hybridized probes while varying temperature over a range of temperatures. The range of temperatures during which fluorescence is observed is 0-60 degrees C. Detecting the target nucleic acid comprises detecting the polymorphic variant sequence. The target nucleic acid is derived from non-human animal or organism, plant, bacteria, fungi, archae, cell or tissue, or preferably from a patient. Detecting a target nucleic acid also comprises: (a) flowing a mixture comprising the target nucleic acid in an analysis channel; (b) flowing at least a first and a second probe into the analysis channel; (c) hybridizing a first probe to the target nucleic acid; (d) hybridizing a second probe to the target nucleic acid, where the second probe hybridizes to the target nucleic acid substantially adjacent to the first probe, and where hybridization of the second probe stabilizes hybridization of the first probe; and (e) detecting the hybridization of the first probe by a non-Sanger detection step. The first and second probes are independently selected from at least two groups of probes. The method further comprises hybridizing a third probe to the target nucleic acid. The first, second and third probes are provided from at least one probe set comprising at least 10 % of all possible nucleic acids of a selected type for a selected length, where the selected length is at least about 5 probe monomers. The first, second or third group of probes comprises at least two probe sizes selected to have an equal Tm. The **probe** monomers comprise one or more of a **nucleotide** or PNA monomer. Detecting a polymorphic variant in a polymorphic nucleic acid sequence comprises: (a) flowing a mixture comprising a polymorphic nucleic acid sequence, at least two probes and a buffer into an analysis channel; and (b) detecting hybridization of at least one of the two probes to determine the identity of the polymorphic variant in the polymorphic nucleic acid sequence by varying temperature within a detection region located at a position along a length of the analysis channel. The mixture comprises a salt in a concentration of 0.2-2, 0.5-1.5 or 0.8-1.2, preferably 1 M. The detecting step comprises measuring a signal intensity resulting from hybridization of the hybridizing probes and the target nucleic acid. The method further comprises providing the analysis channel in a microfluidic device, where the analysis channel comprises one or more detection regions and one or more temperature control region. Preferred Probes: The labels of the members of the first group of probes interact with labels of the second group, when the labels are in proximity to one another. The proximity of the first probe to the second probe stabilizes binding of at least one of the first and second probes. The first and second probes are substantially proximal when hybridized to the target nucleic acid in a mixture comprising a buffer and comprise a fluorescence resonance energy transfer (FRET)FRET pair. The first probe is labeled with a fluorescent reporter moiety at one of its termini, and the second probe is labeled with a quencher moiety at one of its termini, so that upon hybridization of the probes with the target nucleic acid, fluorescence of the reporter moiety is quenched, where fluorescence of the reporter moiety is reduced, or hybridization of the first and second probes with the target nucleic acid causes an increase in fluorescence emission. One of the at least two

groups further comprises a subset of probes having w number of nucleotides, which when present in the first set, w is not equal to x and when present in the second set, w is not equal to y. The first and second groups are components of one or several physical groups and comprise one or more of nucleobase, sugar or internucleotide analog. The first or second group comprises: (a) at least 60, 70, 75, 80, 85, 90 or 95 % of all possible nucleic acid probe sequences having x or y nucleotides; or (b) probes of length w. x = y; x or y = an integer between 5-10, 6-9 or 7-12, or preferably, 7 or 10. A Tm of the probes of the first and/or second group are selected to be approximately equal. The first or second probe comprises at least one promiscuous base, consisting of inosine or azidothimidine. The probes from the second and third groups are differently labeled. The at least one probe from the second group is labeled with a fluorescent reporter dye at one of its termini, and the at least one probe from the third group is labeled with a quencher molecule at one of its termini, so that upon hybridization of the probes from the first, second and third groups with the sample, the fluorescence of the reporter dye is quenched so as to cause a reduction in fluorescence emission of the reporter dye. The nucleobase analogs include covalently bound minor groove binders, intercalators or other modifications that **enhance** hybridization **avidity** or **specificity** of the first or second probes to the target nucleic acid. The minor groove binders comprise DAPI or Hoeschst 33258. The internucleotide analogs comprise one or more of phosphate ester or non-phosphate oligonucleotide analog, which is a PNA. The phosphate ester analogs comprise conformationally restricted nucleotides, alkyl phosphonates, phosphoroamidates, alkylphosphotriesters, phosphorothioates or phosphorodithioates. The target nucleic acid comprises a polymorphic variant sequence. The first probe is fully complementary to the polymorphic variant sequence. The second probe hybridizes substantially adjacent to the polymorphic variant sequence. Preferred Library: The library may also comprise at least 10 % or all possible nucleic acids for a monomer length z, which is at least 5 and does not equal x or y. The nucleic acids of the library comprise non-natural nucleic acid monomers and display greater avidity or specificity for a target nucleic acid than a corresponding natural nucleic acid. The labels of the library comprise one or more fluorescent, luminescent or colorimetric labels. At least 90 % or the 10 % comprises one or more of PNA, LNA or a base-modified nucleic acid. The members are arranged dried on a solid surface in a re-hydrateable form, in substantially separate or overlapping pools, in microtiter wells or in a microfluidic system. They are arranged in a format accessible by the microfluidic system. Preferred System: The system further comprises a detector. The sampling system of the genetic analysis system comprises a pipettor affixed to the microfluidic device, comprising at least two intersecting microscale channels. At least one of the two intersecting channels is an analysis channel. The analysis channel is subjected to an increase or decrease in temperature. The sampling and delivering probes comprise delivering at least three nucleic acid probes from the sources of probes to the vessel. The vessel is in contact with a thermal element, where a region of the vessel is subjected to an increase or decrease in temperature. The nucleic acid probes comprise hybridizing probes and flanking sequences. The hybridizing probes comprise at least one interrogation base.

USE - The method is useful for high throughput genetic analysis. (78 pages)

L3 ANSWER 4 OF 11 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2003-17940 BIOTECHDS
TITLE: Detecting a **nucleotide** target sequence for detecting genetic disease, by using a **helper probe**;
capture DNA **probe** and **helper** DNA **probe** for SNP analysis, genotyping and disease diagnosis

AUTHOR: JACOBSEN N; JAKOBSEN M H; SKOUV J
PATENT ASSIGNEE: EXIQON AS
PATENT INFO: EP 1251183 23 Oct 2002
APPLICATION INFO: EP 2002-388014 18 Feb 2002
PRIORITY INFO: US 2001-284729 18 Apr 2001; US 2001-284729 18 Apr 2001
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-459558 [44]
AN 2003-17940 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - Detecting a nucleotide target sequence using a hybridization mixture comprising a capture oligonucleotide (I) and a **helper probe** oligonucleotide (II) comprising modified sequences, capable of enhancing binding of (I) to the target, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following: (1) a kit comprising (I) and (II); and (2) use of an oligonucleotide for enhancing the capture of a target sequence by (I) comprising modified nucleic acid residues.

BIOTECHNOLOGY - Preferred Method: (I) comprises modified nucleic acid resides, and is immobilized. (II) is capable of reshaping the secondary structure of the target, and invades a double stranded molecule in the region of the target sequence by denaturing bonds between the target and complementary sequences. (I) has higher specificity and affinity for a target than a complementary DNA target nucleotide sequence. (II) has both modified and non-modified nucleic acid residues; more than 50 % are modified, but does not contain more than 6 consecutive modified residues. (II) is a gabmer, and contains 4-100, preferably 8-15) total residues. A modified residue in (I) and (II) is at the 2'-position in the ribose e.g. 2'-deoxy-2'-fluor ribonucleotides, 2'-O-methyl ribonucleotides, 2'-O-methoxyethyl ribonucleotides, peptide nucleic acids, 5-propynyl pyrimidine ribonucleotides, 7-deazapurine ribonucleotides, 2,6-diamiopurine ribonucleotides and 2-thio-pyrimidine ribonucleotides. At least one is an LNA-residue, preferably an oxy-LNA residue. The non-modified residues contain deoxyribonucleotides. The sample is an amplicon (e.g. blood, urine or tissue) prepared from a human or animal sample. The amplicon and (I) are conjugated to a reporter group e.g. biotin, digoxigenin, fluorescent groups, dansyl (5-dimethylamino)-1-naphthalenesulfonyl, DOXYL (N-oxyl-4,4-dimethyloxazolidine), PROXYL (N-oxyl-2,2,5,5-tetramethylpyrrolidine), TEMPO (N-oxyl-2,2,6,6,-tetramethylpiperidine), dinitrophyl, acridines coumrins, Cy3 and Cy5, erythrosine coumaric acid, umbelliferone, Texas red, rhodamine, tetramethylrhodamine, Rox, 7-nitrobenzo-2-oxa-1-diazole (NBD), pyrene, fluorescin, Europium, Ruthenium, Samarium, and rare earth metals, radioisotopic labels, chemiluminescence labels, spin labels, antigens, antibodies, haptens, carrier systems for cell membrane penetration such as fatty acid residues, steroid moieties (cholesteryl), vitamin A, D or E, folic acid, peptides for specific receptors, groups for mediating endocytose, epidermal growth factor (EGF), bradykinin and platelet derived growth factor (PDGF). (I) detects a single nucleotide polymorphism (SNP). Preferred Kit: (I) is immobilized on a substrate platform. (I) is capable of discriminating between target alleles differing b a SNP. The kit further comprises polymerase chain reaction (PCR) primers for amplifying the target sequence; at least one primer is conjugated to a reporter group.

USE - The method and kit are useful for detecting a SNP for genotyping and for diagnosis (claimed). The method and kit are also useful for diagnosis of genetic disease.

ADVANTAGE - The helper oligonucleotides have **enhanced specificity**.

EXAMPLE - An LNA-modified oligonucleotide (ApoE-as-112) (I) was covalently immobilized to the wells of a microtitre plate by UV irradiation an used as a capture probe in a hybridization assay with a complementary double-stranded DNA target. The hybrid was detected by including an LN **helper probe** in the mixture. PCR

reactions were carried out on patient samples (200 ng genomic DNA). The genotype of patients was determined, and sensitivity was increased using the **helper probe**. 5'-TGCACCTCGCCGCGGTAC-3' (I).(24 pages)

L3 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:906543 CAPLUS
DOCUMENT NUMBER: 137:365936
TITLE: Oligonucleotide probes with improved hybridization efficiency and specificity in detection of genetic polymorphisms in arrays
INVENTOR(S): Luo, Yuling; Chen, Anthony C.; Li, Kaijun
PATENT ASSIGNEE(S): Genospectra, Inc., USA
SOURCE: PCT Int. Appl., 59 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002095057	A2	20021128	WO 2002-US16373	20020524
WO 2002095057	A3	20030220		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

US 2002177157	A1	20021128	US 2002-155946	20020524

PRIORITY APPLN. INFO.: US 2001-293666P P 20010524
US 2001-293675P P 20010524

AB The invention provides methods and kits for detecting and/or quantifying nucleic acid sequences of interest by using pairs of probes containing donor-acceptor moieties that when hybridized on a target polynucleotide, with one of the probes being hybridized to a sequence of interest in the target polynucleotide, places the donor-acceptor moieties in sufficiently close proximity such that a detectable signal is generated. Methods of the invention are particularly useful for genotyping anal. and gene expression profiling. Methods of the invention are easily adaptable to arrays and automation. The invention also provides probes with **enhanced** hybridization efficiency and/or **specificity** comprising a probe portion having a spacer element and/or a minor groove binder mol., and methods and kits for using these probes.

L3 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 2002:696538 CAPLUS
DOCUMENT NUMBER: 137:227601
TITLE: Nucleic acid binding enhancement by conjugation with nucleotides, nucleosides, bases and/or their analogs for improved degree and specificity of hybridization
INVENTOR(S): Erikson, Glen H.; Daksis, Jasmine I.
PATENT ASSIGNEE(S): Ingeneus Corporation, Barbados
SOURCE: U.S. Pat. Appl. Publ., 9 pp., Cont.-in-part of U. S. Ser. No. 909,496.
CODEN: USXXCO
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 11

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002127590	A1	20020912	US 2002-80767	20020222
US 6403313	B1	20020611	US 1999-468679	19991221
US 6420115	B1	20020716	US 2000-613263	20000710
US 2002031775	A1	20020314	US 2001-909496	20010720
US 6656692	B2	20031202		
WO 2002024946	A2	20020328	WO 2001-IB1643	20010910
WO 2002024946	A3	20030313		
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
AU 2001084350	A5	20020402	AU 2001-84350	20010910
EP 1319088	A2	20030618	EP 2001-963329	20010910
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
JP 2004520815	T2	20040715	JP 2002-529538	20010910

PRIORITY APPLN. INFO.:			
	US 1999-468679	A2	19991221
	US 2000-613263	A2	20000710
	US 2000-664827	A2	20000919
	US 2001-281547P	P	20010404
	US 2001-909496	A2	20010720
	WO 2001-IB1643	W	20010910

AB An improved method of forming a specific complex between a probe containing probe nucleobases and a target containing target nucleobases, includes mixing the probe and the target under hybridizing conditions, wherein the **probe** and/or the target is conjugated to a **blocking** agent, which **enhances** the **avidity** and/or **specificity** of hybridization, whether by Watson-Crick motif or by homologous binding motif. The blocking agent contains at least one nucleobase and can be, e.g., a free nucleobase, a nucleoside or a nucleotide. Conjugation enhances hybridization by hindering the probe and/or target from existing in a conformation antithetical to hybridization. Thus, reaction of 2 pmoles of a 15-mer single-stranded DNA probe containing 6 adenine bases (conjugated with 3 pmoles of thymine) with 2 pmoles of a wild-type antisense strand (50-mer) in the presence of YOYO-1 results in 78% increased formation of parallel homologous complexes between perfectly homologous sequences. By contrast, the efficiency of formation of parallel homologous complexes containing a 1-bp A-G mismatch was increased by only 16% when the probe was conjugated 25% with thymine.

L3 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

ACCESSION NUMBER:	2002:453552 CAPLUS
DOCUMENT NUMBER:	137:197218
TITLE:	Nucleobase amino acids incorporated into the HIV-1 nucleocapsid protein increased the binding affinity and specificity for a hairpin RNA
AUTHOR(S):	Takahashi, Tsuyoshi; Ueno, Akihiko; Mihara, Hisakazu
CORPORATE SOURCE:	Dep. of Bioengineering, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Yokohama, 226-8501, Japan
SOURCE:	ChemBioChem (2002), 3(6), 543-549 CODEN: CBCHFX; ISSN: 1439-4227
PUBLISHER:	Wiley-VCH Verlag GmbH
DOCUMENT TYPE:	Journal
LANGUAGE:	English

AB L-α-Amino acids with a nucleobase in the side chain (nucleobase amino acids; NBAs) were used to enhance the function of RNA-binding proteins that recognize structured RNA. These NBAs were utilized in the three-dimensional structure of the protein to **enhance** RNA binding affinity and **specificity** as a result of selective recognition of NBAs by RNA bases. NBA units were incorporated at various positions into the HIV-1 nucleocapsid protein NCp7 (residues 1-55), which contains two CCHC-type (Cys-X2-Cys-X4- His-X4-Cys-type; X = an amino acid residue) zinc knuckle domains. The binding ability was evaluated by using the stem-loop (SL)3 region of HIV-1 Ψ-RNA. Visible light absorption measurements revealed that two zinc ions bound strongly and quant. to the NBA-NCp7 mol. and to the wild-type NCp7 protein. This result indicates that the incorporation of NBA units composed of L-α-amino acids did not influence the formation of the specific structure of NCpZ Binding anal. with fluorescein-labeled SL3 RNA revealed that incorporation of NBA units into the NCp7 protein at appropriate positions increased its RNA binding affinity and specificity. An NBA-NCp7 protein that possessed cytosine and guanine NBA units at positions 13 and 46, resp., showed a binding affinity for SL3 RNA ninefold higher than that of wild-type NCp7 as a result of the specific and cooperative interaction of the NBA units with RNA bases. These results clearly demonstrate that inclusion of NBA units in the three-dimensional structure of an RNA-binding protein is a useful strategy for enhancing the function of the protein.
REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 8 OF 11 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1999:467997 BIOSIS
DOCUMENT NUMBER: PREV199900467997
TITLE: PNA **blocker** probes **enhance** **specificity** in **probe** assays.
AUTHOR(S): Fiandaca, Mark J. [Reprint author]; Hyldig-Nielsen, Jens J. [Reprint author]; Coull, James M. [Reprint author]
CORPORATE SOURCE: Boston Probes, Inc., 75E Wiggins Avenue, Bedford, MA, 01730, USA
SOURCE: Nielsen, P. E. [Editor]; Egholm, M. [Editor]. (1999) pp. 129-141. Peptide nucleic acids: Protocols and applications. print.
Publisher: Horizon Scientific Press, P. O. Box 1, Wymondham, Norfolk NR18 0EH, England, UK; Horizon Press Inc., 156 Fifth Ave., New York, New York 10010, USA.
ISBN: 1-898486-16-6.
DOCUMENT TYPE: Book; (Book Chapter)
LANGUAGE: English
ENTRY DATE: Entered STN: 9 Nov 1999
Last Updated on STN: 9 Nov 1999

L3 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1999:501539 CAPLUS
DOCUMENT NUMBER: 131:282104
TITLE: PNA **blocker** probes **enhance** **specificity** in **probe** assays
AUTHOR(S): Fiandaca, Mark J.; Hyldig-Nielsen, Jens J.; Coull, James M.
CORPORATE SOURCE: Boston Probes, Inc., Bedford, MA, 01730, USA
SOURCE: Peptide Nucleic Acids (1999), 129-141. Editor(s): Nielsen, Peter E.; Egholm, Michael. Horizon Scientific Press: Norfolk, UK.
CODEN: 67YLA6
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Non-labeled PNA "blocker" probes were used to prevent mismatch hybridization of labeled probes to non-target sequences. The use of PNA blockers significantly decreased unwanted hybridization without a

corresponding decrease in the sensitivity of detection of complementary targets. Furthermore, PNA probes and blockers provided higher signal to noise ratios than corresponding probes and blockers made of DNA. As a result, following PCR amplification, it was possible to detect a single base mutation in the K-ras gene at levels of only 1.5 copies per 100 copies of wild type DNA.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L3 ANSWER 10 OF 11 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 1

ACCESSION NUMBER: 1998024538 EMBASE
TITLE: Multicolor molecular beacons for allele discrimination.
AUTHOR: Tyagi S.; Bratu D.P.; Kramer F.R.
CORPORATE SOURCE: S. Tyagi, Department of Molecular Genetics, Public Health Research Institute, 455 First Ave., New York, NY 10016, United States. sanjay@phri.nyu.edu
SOURCE: Nature Biotechnology, (1998) 16/1 (49-53).
Refs: 12
ISSN: 1087-0156 CODEN: NABIF
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Molecular beacons are hairpin-shaped oligonucleotide probes that report the presence of specific nucleic acids in homogenous solutions. When they bind to their targets they undergo a conformational reorganization that restores the fluorescence of an internally quenched fluorophore. We found that their hairpin conformation enables the use of a wide variety of differently colored fluorophores. Using several molecular beacons, each designed to recognize a different target and each labeled with a different fluorophore, we demonstrate that multiple targets can be distinguished in the same solution, even if they differ from one another by as little as a single **nucleotide**. A comparison of '**hairpin** probes' with corresponding 'linear probes' confirms that the presence of the hairpin stem in molecular beacons significantly **enhances** their **specificity**.

L3 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1988:565002 CAPLUS
DOCUMENT NUMBER: 109:165002
TITLE: Dot blot detection of point mutations with adjacently hybridizing synthetic oligonucleotide probes
AUTHOR(S): Alves, A. M.; Carr, F. J.
CORPORATE SOURCE: Pharm. Div., ICI, Macclesfield/Cheshire, SK10 4TG, UK
SOURCE: Nucleic Acids Research (1988), 16(17), 8723
CODEN: NARHAD; ISSN: 0305-1048
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A simple method is described that **enhances** the hybridization **specificity** of oligonucleotide probes for dot blot anal. Gels are hybridized with a 32P-labeled oligonucleotide **probe**, together with an adjacently hybridizing **nucleotide**, for 2 h. Then the gels are immersed in ligase solution (0.3 units/μL T4 DNA ligase) for 80 min. at 16° before washing. The ligase treatment resulted in the removal of the background high-mol.-weight hybridization signal and a specific dot blot hybridization of the probe to target DNA alone. This method was used to detect the activating mutation in the Ha-ras gene in human T24 bladder carcinoma cells.

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L16: Entry 6 of 16 File: PGPB Dec 26, 2002

DOCUMENT-IDENTIFIER: US 20020197630 A1
TITLE: Systems and methods for high throughput genetic analysis

Detail Description Paragraph:
[0072] The first and second groups of probes can be components of a single physical group, or can be components of a plurality of physical groups. The first or second probes can comprise at least one promiscuous base. For example, the at least one promiscuous base is selected from a group consisting of: inosine and azidothimidine. The first or second probes can comprise one or more of: a nucleobase analog, a sugar analog or an internucleotide analog. The nucleobase analogs can include covalently bound minor groove binders or intercalators that enhance hybridization avidity or specificity of the nucleic acid probes to a target. The internucleotide analogs can comprise one or more of: a phosphate ester analog and a non-phosphate oligonucleotide analog, for example. For example, the non-phosphate oligonucleotide analog is a PNA. The phosphate ester analogs can be selected from a group consisting of conformationally restricted nucleotides, alkyl phosphonates, phosphoroamidates, alkylphosphotriesters, phosphorothioates and phosphorodithioates.

CLAIMS:

23. The method of claim 15, wherein the nucleobase analogs include covalently bound minor groove binders or intercalators that enhance hybridization avidity or specificity of the first or second probes to the target nucleic acid.

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PNA **blocker** probes **enhance** **specificity** in **probe** assays

AUTHOR(S): Fiandaca, Mark J.; Hyldig-Nielsen, Jens J.; Coull, James M.
CORPORATE SOURCE: Boston Probes, Inc., Bedford, MA, 01730, USA
SOURCE: Peptide Nucleic Acids (1999), 129-141. Editor(s): Nielsen, Peter E.; Egholm, Michael. Horizon Scientific Press: Norfolk, UK.
CODEN: 67YLA6
DOCUMENT TYPE: Conference
LANGUAGE: English
AB Non-labeled PNA "blocker" probes were used to prevent mismatch hybridization of labeled probes to non-target sequences. The use of PNA blockers significantly decreased unwanted hybridization without a corresponding decrease in the sensitivity of detection of complementary targets. Furthermore, PNA probes and blockers provided higher signal to noise ratios than corresponding probes and blockers made of DNA. As a result, following PCR amplification, it was possible to detect a single base mutation in the K-ras gene at levels of only 1.5 copies per 100 copies of wild type DNA.